Effects of Hyaluronic Acid and Other Glycosaminoglycans on Fibrin Polymer Formation†

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ABSTRACT: Previously, we reported that the glycosaminoglycan (GAG) hyaluronic acid (HA) specifically bound to the plasma protein fibrinogen [LeBoeuf, R. D., Raja, R. R., Fuller, G. M., & Weigel, P. H. (1986) *J. Biol. Chem. 261*, 12586]. The binding of other macromolecules to fibrinogen could influence the conversion of fibrinogen to fibrin. Therefore, we tested whether HA and other GAGs could alter the kinetics of fibrin polymer formation and the physical structure of the resulting gel. In this study, we present data showing that the GAGs HA and chondroitin sulfate (CS) affect fibrin formation in three specific ways: (i) they decreased the clotting time of fibrinogen 3–10-fold; (ii) both GAGs increase significantly the rate of fibrin polymer formation; and (iii) fibrin gels containing HA or CS had a final A_{450} that was greater than controls, indicating that these two glycosaminoglycans influence either the final size of fibrin fibrils or the extent of the lateral association between fibrils. These results demonstrate that the interactions of HA and CS with forming fibrin polymers can alter both the kinetics of formation and may produce structural changes in fibrin gels.

Fibrin formation is the critical event in hemostasis and is essential for the maintenance and repair of vascular tissues (Doolittle, 1984). Previous studies of fibrin in formation have focused on understanding the molecular mechanisms involved in the assembly of monomeric fibrin into polymers and protofibrils. These studies have established that pH and ionic strength (Ferry & Morrison, 1947; Blomback & Okada, 1982) and calcium concentration (Boyer et al., 1972; Endres & Scheraga, 1972; Marguerie et al., 1979) can alter the rate of fibrin polymer formation and the physical structure of fibrin. Although these investigations have been instrumental in providing meaningful insights into the molecular mechanisms involved in the assembly of fibrin gels in vitro, they do not address factors that may influence in vivo fibrin formation. This is so because, with the exception of pathophysiological conditions, pH and ionic perturbations in vivo are generally rapidly restored. Therefore, the observed effects of pH, ionic strength, and Ca^{2+} concentration on fibrin formation would not normally influence fibrin assembly in vivo.

A more appropriate model of in vivo fibrin formation must consider molecules that may interact with fibrin(ogen) during coagulation. Therefore, recent studies examining factors that may influence in vivo fibrin have focused on components of the subendothelial basal lamina. Extracellular matrix molecules are likely components which may influence fibrin polymer formation since, depending on the type and extent of the vascular injury, they will come in contact with fibrin as it is forming. The rationale for these studies is that, after the activation of the coagulation cascade, the rate of fibrin formation and the type of fibrin formed may be dependent in part on the interaction of fibrin(ogen) with components of the basal lamina. Studies of fibrin(ogen) interaction with constituents of the extracellular matrix (ECM)[1] should provide insights into factors that may affect the formation and localization of fibrin in vivo. Investigations showing that plasma fibronectin (pFbn) is cross-linked to fibrin networks and that pFbn also alters the physical structure of fibrin gels (Kamykowski et al., 1981; Chow et al., 1983; Okada et al., 1985) suggest that further examinations of the potential importance of ECM components on fibrin formation are warranted.

Recently, we have shown that fibrinogen specifically binds the glycosaminoglycan HA and that the sulfated GAG CS competes weakly with HA for fibrinogen binding (LeBoeuf et al., 1986). Whether the specific interactions between fibrinogen and HA could influence the formation of fibrin was tested in this study. We determined the effects of the glycosaminoglycans HA, CS, and heparin (HP) on the rate of fibrin polymer formation and on the physical structure of fibrin.

In this report, we provide evidence showing that two glycosaminoglycans, HA and CS, alter the rate of fibrin polymer formation and the apparent size of fibrin fibrils, whereas HP had no effect. The results from this study reinforce the concept that ECM components may be critically important in the formation of a physiological clot which in turn could also affect the rate and extent of reparative processes (Weigel et al., 1986).

Experimental Procedures

Materials. Hyaluronic acid, ovine testicular hyaluronidase, chondroitin sulfate (mixed isomer of 4- and 6-sulfate, grade III), dextran sulfate (M_r 8000), glucuronic acid, *N*-acetylglucosamine, and dextran (M_r 40 000) were obtained from Sigma Chemical Co. (St. Louis, MO). Highly sulfated heparin was obtained from V-Labs (Covington, LA). Bovine thrombin

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[1] Abbreviations: GAG, glycosaminoglycan; ECM, extracellular matrix; HA, hyaluronic acid; CS, chondroitin sulfate; HP, heparin; pFbn, plasma fibronectin; BSA, bovine serum albumin; A_{450}, absorbance at 450 nm; PBS, phosphate-buffered saline; HEPES, *N*-(2-hydroxyethyl)-piperazine-*N*'-2-ethanesulfonic acid.

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(pharmaceutical grade) was obtained from Parke-Davis (Morris Plains, NJ). Human fibrinogen was prepared from outdated blood plasma by ammonium sulfate and ethanol precipitation followed by DEAE-cellulose ion-exchange chromatography (Mosher & Blount, 1973). Purity was assessed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Laemmli, 1970) and by a clotting assay. Fibrinogen used in all experiments was homogeneous on polyacrylamide gels and >98% clottable. Human and plasma fibronectin (pFbn) was provided by Dr. Jo Ellen Schweinle of the University of Texas Medical Branch. All polysaccharides, except HA, were used as supplied by the vendor. HA was purified by cetylpyridinium chloride fractionation on Celite and ethanol precipitation (Scott, 1960), and purity was >95%. Hyaluronic acid oligosaccharides were prepared by partial hyaluronidase digestion (Raja et al., 1984) or by sonication of high molecular weight HA. Oligosaccharide size was determined from the ratio of total sugar to reducing sugar.

Measurements of Fibrin Formation. Reaction mixtures of fibrinogen or of fibrinogen and GAG were placed in cuvettes, and the A_{450} was set to zero; the solutions were then removed, and bovine thrombin was added to these solutions during vortexing. The clotting solutions were then immediately placed in cuvettes, and fibrin polymer formation was monitored by recording the changes in A_{450} over a 1-h interval. Some clotting solutions were monitored for up to 2 h and differed from the 1-h A_{450} reading by <15%. Unless noted otherwise, final A_{450} values reported were recorded at 60 min. Clotting reactions using low thrombin concentrations show a period in which the A_{450} does not change (lag phase) which is followed by a rapid increase in A_{450} (Okada et al., 1985). The clotting time of fibrin(ogen) solutions was taken as the inflection point in the absorbance curve that occurred between the lag phase and the rapid increase in A_{450}.

General Information. Absorbance measurements were made on a Gilford 250 spectrophotometer. Unless noted otherwise, all reagents were dissolved in 10 mM HEPES, 150 mM NaCl, 7 mM KCl, and 2 mM $CaCl_2$ and $MgCl_2$, pH 7.4, which was designated HEPES buffer. Glucuronic acid was quantitated by a phenol–sulfuric assay (McKelvy & Lee, 1964), and reducing sugar content was determined by the procedure of Dygert et al. (1965). Protein determinations were performed by the method of Bradford (1976) using either BSA or fibrinogen as standards. Polyacrylamide gel analyses were performed with 7.5% and 10% polyacrylamide gels in the presence of 6 M urea according to the procedures of Laemmli (1970). Gel samples were heated at 100 °C for 5 min in the presence of 11 mM dithiothreitol. After reduction, free sulfhydryl groups were alkylated with 55 mM iodoacetamide. Proteins bands on gels were visualized with $AgNO_3$ according to published procedures (Merril et al., 1981).

Data Analysis. The initial rate of fibrin polymer formation was determined from changes in A_{450} during clotting using the linear portion of each absorbance profile on untransformed data. The data from each profile were analyzed by least-squares regression analysis (Sokal & Rohlf, 1981). For all the data presented, the initial rates of fibrin in polymer formation were significantly different from zero ($p < 0.05$), and each set of data points fit a linear model ($r = 0.99$–0.95). Comparisons of the initial rate of fibrin polymer formation for experimental and control polymerizing fibrin solutions were done by using an F test for comparisons of the regression coefficient (Sokal & Rohlf, 1981). Determinations of the maximum initial rate of fibrin polymer formation and of the final A_{450} were determined by regression analysis after appropriate transformations of the data to make them fit a linear model.

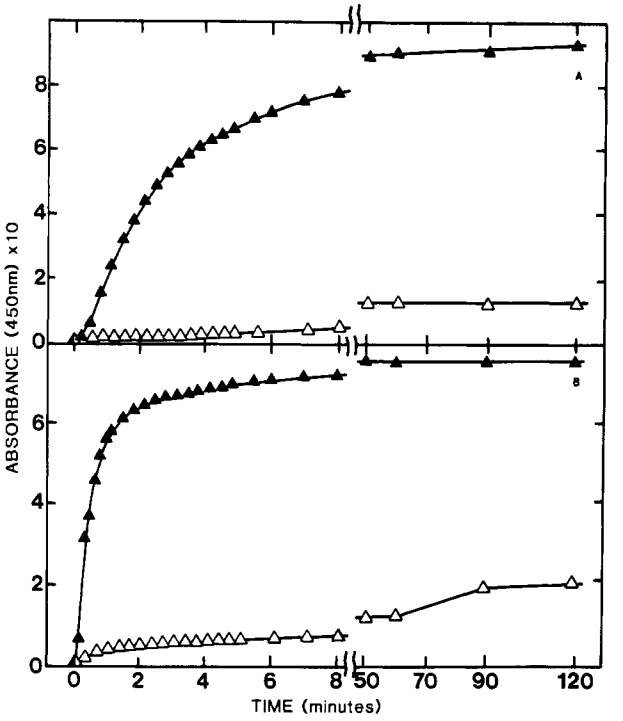

FIGURE 1: Effects of HA on fibrin in polymer formation. Fibrinogen (600 μg/mL) with and without 2.5 mg/mL HA was clotted with 0.6 NIH unit of thrombin, and the A_{450} for each solution was determined for 120 min. Hyaluronic acid and fibrinogen were mixed together and preincubated for 60 min prior to the addition of thrombin (A), or HA and fibrinogen were mixed and then thrombin was added immediately (B). (▲) HA present; (△) control.

RESULTS

Effects of HA on the Rate of Fibrin Polymer Formation. We have recently demonstrated that fibrinogen specifically binds HA (LeBoeuf et al., 1986); therefore, initial experiments on the effects of GAGs on the formation of fibrin were performed by adding HA to fibrinogen solutions 60 min prior to the addition of thrombin. Fibrin formed in the presence of HA polymerized up to 15 times more rapidly than control fibrinogen solutions (Figure 1A). Statistical comparisons of the rate of fibrin polymer formation within each experiment showed that the initial rate of fibrin polymer formation in HA–fibrinogen solutions was significantly greater than the initial rate of control fibrin solutions ($p < 0.05$). Moreover, the final turbidity of fibrin fibrils in HA–fibrin solutions was as much as 8 times greater than control fibrin gels.

To determine whether preincubation of HA with fibrinogen was required for the observed effects of HA on fibrin polymer formation, HA (2.5 mg/mL) and fibrinogen (1.0 mg/mL) were mixed together, and 0.6 NIH unit of thrombin was added to this solution immediately after mixing. The initial rate of fibrin polymer formation was not significantly different ($p > 0.05$) between fibrinogen solutions preincubated with HA and fibrinogen solutions not preincubated with HA (Figure 1A,B). However, preincubation of fibrinogen solutions with HA produced fibrin gels that had a final A_{450} about 25% greater than the fibrinogen solution that was not preincubated.

Changes in Ca^{2+} concentration (Boyer et al., 1972; Endres & Scheraga, 1972; Marguerie et al., 1979) are known to alter the kinetics and type of fibrin formed. The effects of HA on fibrin gel structure were observed whether or not 2 mM $CaCl_2$ was present (data not shown). The self-association of HA or the association of HA and thrombin into a gellike matrix was

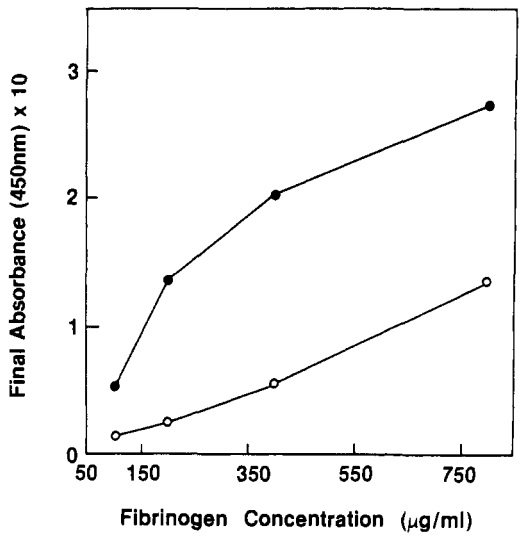

FIGURE 2: Effects of initial fibrinogen concentration of HA-induced changes in fibrin polymer formation. The initial concentration of fibrinogen in clotting reactions was titrated from 100 to 800 μg/mL, and the final A_{450} was determined for control and HA-containing fibrin gels. Thrombin and HA (M_r 32 200) concentrations were 0.04 NIH unit/mL and 2.5 mg/mL, respectively. Fibrinogen concentration was regressed on final A_{450}, and slopes of the regression lines for fibrin and HA–fibrin gels were 1.8×10^{-2} and 2.9×10^{-2} A_{450} units/100 μg of fibrinogen. (○) Control; (●) HA present.

not responsible for the observed changes in fibrin polymer formation since HA alone or HA and thrombin solutions had no absorbance at 450 nm after 60 min at room temperature. HA did not affect the amount of fibrinogen converted to fibrin. The amount of fibrinogen remaining in solution 120 min after the addition of thrombin differed by <10% between HA and control fibrin solutions. Additionally, the effects of HA on fibrin polymer formation were independent of the initial concentration of fibrinogen (Figure 2). At each fibrinogen concentration tested, the final A_{450} of HA–fibrin gels was greater than that of control fibrin gels. Moreover, the change in the final $A_{450}/100$ μg of fibrinogen was not significantly different between control and HA-containing fibrin gels.

Previous studies have shown that globular proteins in solution with polysaccharides, such as HA and dextran, can be excluded from the solvent and compartmentalized within "holes" of the polysaccharide matrix and that this effect is determined largely by the concentration and size of the polysaccharide [reviewed in Comper and Laurent (1978)]. If a similar situation occurred in HA–fibrin(ogen) solutions, the effective concentration of fibrinogen would increase which could change the kinetics of fibrin polymer formation. The possibility that the effects of HA on fibrin polymer formation were due to compartmentalization was tested by clotting fibrinogen in the presence of HA (M_r 32 500) or dextran (M_r 40 000) at 2.5 mg/mL. If the effects of HA on fibrin polymer formation were due to compartmentalization or nonspecific exclusion effects, then dextran should also affect fibrin polymer formation. Fibrin polymer formation in the presence of dextran was unaltered, while HA containing clotting solutions showed the previously described effects, indicating that HA effects on fibrin polymer formation were not due to fibrinogen exclusion from solution by HA.

Effects of Other GAGs and Saccharides on Fibrin Polymer Formation. To determine the specificity of effects of GAGs on fibrin formation, we measured (i) the clotting time, (ii) the initial rate of fibrin polymer formation, and (iii) the final A_{450} of fibrin gels in the presence of several saccharides. The addition of HA and CS to clotting solutions altered the three

Table I: Effects of GAG and Carbohydrates on Fibrin Formation[a]

treatment	clotting time (s)	final A_{450}	A_{450}/s
control	300	0.159	1.2×10^{-4} (± 0.20)
hyaluronic acid	30	0.300	25.4×10^{-4} (±3.13)
chondroitin sulfate	100	0.272	5.4×10^{-4} (±0.82)
dextran sulfate	360	0.189	2.2×10^{-4} (±0.42)
heparin	300	0.195	2.4×10^{-4} (±0.70)
glucuronic acid	300	0.069	0.6×10^{-4} (±0.14)
N-acetylglucosamine	300	0.063	0.8×10^{-4} (±0.14)

[a] Fibrinogen (250 μg/mL) was clotted with thrombin (0.08 NIH unit/mL) in the presence of the following: HA, CS, HP, dextran sulfate, glucuronic acid, and *N*-acetylglucosamine. The concentration of the polysaccharides was 2.5 mg/mL, and the monosaccharides were at 15 mg/mL. The A_{450} was recorded for control and experimental fibrin-forming solutions, and the clotting time, final A_{450}, and initial rate of fibrin polymer formation determined for each treatment. Standard errors for A_{450}/s were determined from the standard error for each regression coefficient.

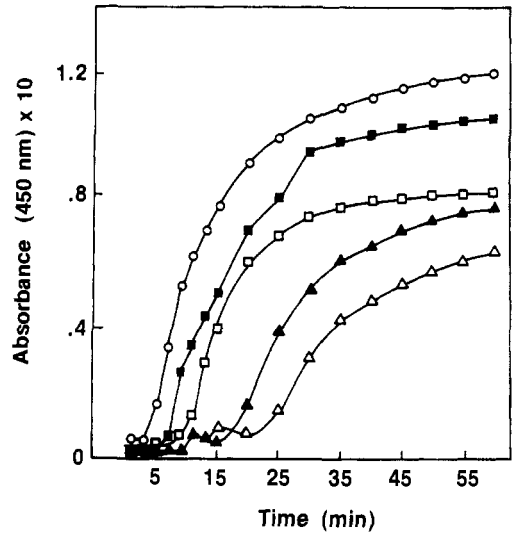

FIGURE 3: Effects of HA concentration on fibrin formation. Absorbance profiles for fibrinogen solutions (250 μg/mL) that were clotted with 0.04 NIH unit of thrombin in the presence of varying HA concentrations. Control (△); HA, 3.8 mg/mL (○); HA, 2.5 mg/mL (■); HA, 1.0 mg/mL (□); HA, 0.5 mg/mL (▲).

parameters whereas dextran sulfate and HP did not (Table I). Glucuronic acid and *N*-acetylglucosamine did not affect clotting time and the rate of polymer formation, but they did lower the final A_{450}. At the concentrations tested, HA was most effective in influencing the clotting time and the final A_{450} followed by CS. This order of influence was also observed in the initial rate of fibrin formation with HA–fibrinogen solutions having a significantly greater rate than CS–fibrinogen solutions ($p < 0.05$). Moreover, the initial rate of increase in fibrin polymer formation in the presence of HA or CS was significantly greater than the rate in control solutions, while the rate of fibrin polymer formation in the presence of the other saccharides was not significantly different from controls.

Effects of HA and CS Concentration on Fibrin Formation. The concentrations of HA and CS in solutions of polymerizing fibrin were titrated over a 30-fold range (0.125 and 3.75 mg/mL) to determine the effects of HA and CS concentration on fibrin polymer formation. Results presented in Figure 3 show fibrin solutions in the presence of varying concentrations of HA.

The specific effects of changing HA concentration on fibrin formation can be summarized as follows: (i) The initial rate of fibrin polymer formation (A_{450} per second) and the final A_{450} are directly related to HA concentration (Figure 4A,B). (ii) The clotting time of fibrinogen solutions is inversely related

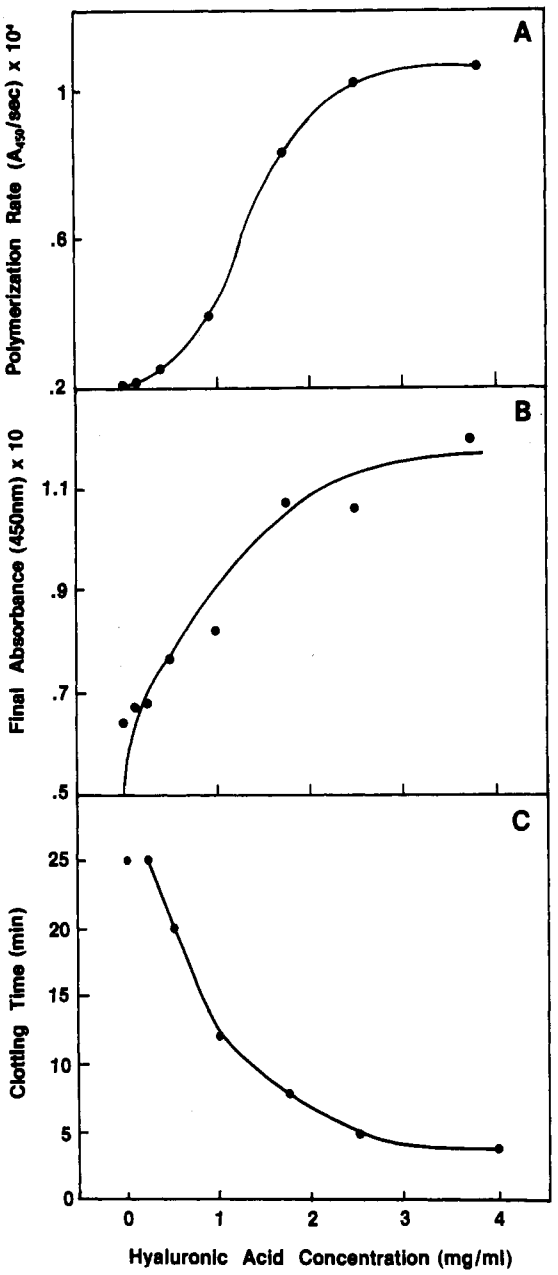

FIGURE 4: Effects of HA concentration on the initial rate of fibrin polymer formation, final A_{450}, and clotting time. Fibrinogen (100 μg/mL) was added to solutions containing 3.75, 2.5, 1.75, 1.04, or 0.520 mg/mL HA (M_r 32 220) and clotted with thrombin (0.04 NIH unit/mL). The rate of change in A_{450} over 60 min was recorded for each HA concentration tested. (A) initial rate of fibrin formation for each HA concentration; (B) final A_{450} for each HA concentration; (C) clotting time for each HA concentration tested.

to HA concentration (Figure 4C). Additional information on the role of HA and CS on fibrin polymer formation was obtained from the data in Figure 4 after reciprocal or log transformation of the original data. This mathematical replot allows one to extrapolate (i) maximum rate constants and (ii) the concentration of HA required to obtain these rates. When the data were treated in this way, it showed that for every 0.5 mg/mL increase in HA concentration, the initial rate of fibrin polymer formation increased by 1.31×10^{-5} A_{450}/s. The predicted maximum rate is 1.07×10^{-4} A_{450}/s and would have occurred at an HA concentration of 11.99 mg/mL. Varying the HA concentration between 2.5 and 0.5 mg/mL resulted in an increase of 0.016 A_{450} in the final A_{450} for every 0.5 mg/mL added to the reaction mixture. Clotting time data were also analyzed in this manner except HA concentration

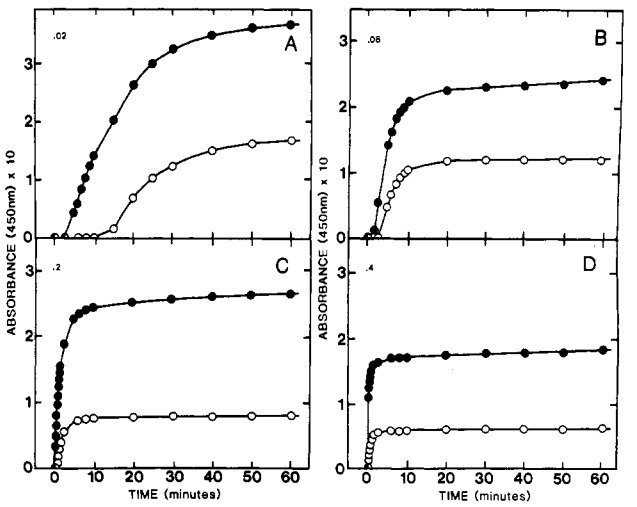

FIGURE 5: Effects of thrombin concentration on the ability of HA to influence fibrin formation. Absorbance profiles for fibrinogen solutions (250 μg/mL) that were formed with and without 2.5 mg/mL HA (M_r 32 200) at the following thrombin concentrations: (A) 0.02, (B) 0.04, (C) 0.2, and (D) 0.4 NIH units/mL. (○) Control; (●) HA present.

(micromolar) log-transformed. These results showed that there was a decrease of 0.88 s in the clotting time for each micromolar increase in HA concentration. Additionally, it should be noted that HA concentrations at or below 0.5 mg/mL did not greatly affect the three measured parameters of fibrin polymer formation.

Chondroitin sulfate influenced formation in a manner qualitatively similar to HA in that it caused (i) a decrease in clotting time, (ii) an increase in the final A_{450} of fibrin gels, and (iii) an increase in the initial rate of fibrin polymer formation. Additionally, the effects of CS on fibrin polymer formation were also dependent on CS concentration. Increases in CS concentration also caused a decrease in clotting time (data not shown). Additionally, the final A_{450} of fibrin gels increased with increasing CS concentration. Regression analysis of these data indicated that for every 0.5 mg/mL increase in CS concentration there was an increase of 0.014 A_{450} unit in the final A_{450} of fibrin gels. Statistical comparisons of the effects of CS concentration on the final A_{450} of fibrin gels to that for HA showed that they were not significantly different ($p < 0.05$).

Effects of HA on Fibrin Formation at Varying Thrombin Concentrations. Changes in thrombin concentration are known to alter the kinetics of fibrin polymer formation and type of fibrin gel formed (Blomback & Okada, 1982). Increases in thrombin concentration in clotting solutions are reported to cause a decrease in clotting time and the turbidity of the fibrin formed. Since we had observed that HA also reduced clotting time when the thrombin concentration was kept constant, we examined the role of varying the concentration of thrombin on the formation of fibrin in the presence of HA. At thrombin concentration between 0.02 and 0.8 NIH unit/mL, the effects of HA on fibrin polymer formation were independent of thrombin concentration (Figure 5A–D). As expected, the clotting time and the final A_{450} of control solutions decreased with increasing thrombin concentration. However, the effects of HA on clotting time and final A_{450} were observed at all thrombin concentrations tested. At two lower thrombin concentrations (0.02 and 0.08 NIH unit/mL), where clotting time was determined, the clotting time in HA-containing solutions was less than that in control solutions. Moreover, the final A_{450} of HA-containing solutions was greater in all cases than in controls (Table II).

Table II: Final A_{450} of HA-Containing and Control Fibrin Gels[a]

[thrombin] (NIH units/mL)	final A_{450}	
	control	+HA
0.02	0.245	0.636
0.04	0.233	0.441
0.08	0.181	0.370
0.16	0.157	0.247
0.24	0.154	0.332
0.28	0.143	0.267
0.40	0.089	0.188
0.80	0.062	0 .176

[a] Fibrinogen solutions (250 μg/mL) with and without 2.5 mg/mL HA (M_r 32 200) were clotted with varying concentrations of thrombin, and the final A_{450} for each fibrin gel was determined.

Discussion

The addition of HA or CS to solutions of fibrinogen prior to clotting with thrombin produced changes in the rate of fibrin polymerization and in the physical structure of fibrin networks. Specifically, the presence of HA or CS in clotting solutions caused increases in turbidity that begin much sooner (decreased clotting time) and continue at a greater rate (polymerization rate) than control clotting solutions (Figure 1). Both of these factors resulted in HA- or CS-fibrin solutions achieving maximum turbidity more rapidly than controls, which we interpret as an acceleration in the rate of fibrin polymer formation. Not only did fibrinogen solutions containing HA and CS form more rapidly but also they had a turbidity that was much greater than controls. Changes in fibrin polymer formation were not altogether unexpected since we had previously shown that there is a specific association between fibrin(ogen) and HA (LeBoeuf et al., 1986). What was unexpected was the extent to which these GAGs altered turbidity and hence fibrin polymer formation.

The effects of HA and CS on fibrin polymer formation were specific since neither HP nor dextran sulfate, the monosaccharides glucuronic acid and *N*-acetylglucosamine, influenced fibrin polymer formation. The extensive changes seen in the fibrin polymer formation were not due to Ca^{2+} since we observed HA and CS changes in fibrin polymer formation in the presence and absence of Ca^{2+}. Moreover, HA was just as effective in altering fibrin polymer formation as the more charged GAG, CS (Comper & Laurent, 1978), indicating that simple electrostatic interactions, between the negatively charge polysaccharide and positively charged groups on the protein, would not fully explain the above observations.

Fibrin gel formation is a two-step process involving first activation and then polymerization/association to produce a fibrin gel. The type of fibrin networks formed is determined primarily from reaction kinetics and not simply the equilibria steps of assembly (Hantgan & Hermans, 1979; Blomback & Okada, 1982). The findings reported here indicate that HA and CS alter the kinetics of fibrin polymer formation by (i) decreasing the clotting time and (ii) increasing the initial rate of fibrin monomer polymerization. These changes in the rate of fibrin polymer formation and the decrease in clotting time in the presence of HA and CS may be due in part to changes in the catalytic and polymerization phases of fibrin formation, or they could be due to nonspecific volume exclusion effects from the addition of these macromolecules. The latter possibility cannot be totally excluded with the present results. However, since the addition of dextran sulfate (M_r 40 000) of equivalent size to HA did not affect fibrin formation, nonspecific exclusion effects, if present, do not appear to be the only mechanism affecting fibrin gel formation in the presence of HA.

Previous studies using fibrinogen–thrombin solutions have shown that clotting time is directly related to the rate of release of fibrinopeptides A and B (Blomback & Okada, 1982). The reduction in clotting time observed in HA- and CS-fibrin may be due to their affecting the rate of fibrinopeptide release. However, correlations of fibrinopeptide release with decreases in clotting time in HA- and CS-fibrinogen solutions would have to be made to verify whether they are accelerating the thrombin-catalyzed release of fibrinopeptides.

A decrease in clotting time and the concomitant increase in the initial rate of fibrin polymer formation by HA or CS could be due to their ability to alter the kinetics of fibrin monomer polymerization (end to end polymerization) and/or protofibril formation (lateral association). To test whether HA could alter the kinetics of fibrin formation independent of the thrombin activation step, we prepared two types of fibrin monomers; one in which both fibrinopeptides are cleaved out and one missing only fibrinopeptide A (Fp A). When these monomers were allowed to assemble in the presence of the GAGs, HA and CS again increased the rate of fibrin polymer formation (end to end polymerization) as well as the formation of fibrin fibrils (lateral association). These studies are consistent with the notion that the two GAGs alter the kinetics of fibrin polymerization.[2,3]

Increases in the turbidity of fibrin gels have been shown to be due an increase in the fibrin fibril mass:length ratio (thick fibers or "coarse clots" (Ferry & Morrison, 1947; Okada et al., 1985; Shah et al., 1982; Fowler et al., 1981). Turbidity measurements have been used to estimate the mass:length ratio of fibrin fibrils (Carr & Hermans, 1978), and these estimates correlate well with other independent measures of fibril size (Carr et al., 1977). For example, there is a direct relationship between fibril size and the extent of turbidity in fibrin gels alone or fibrin formed in the presence of other macromolecules (Torbet, 1986), including the polymeric carbohydrate dextran (Carr & Gabriel, 1980). Thus, increases in turbidity observed in the experiments with HA and CS most probably indicate an increase in size of the fibrin fibril. This increase in fibril size could be due to an increase in the number of fibrin polymers in each fibril or to the direct association of HA with fibrils.

The network structure of fibrin fibers can be altered by a variety of conditions (Ferry & Morrison, 1947; Carr et al., 1977; Hantgan & Hermans, 1979). For example, fibrin formed in high salt (0.5 M NaCl) formed this fibers and took 12 times longer to form than fibrin formed in low salt (0.1 M NaCl) which had thick fibers (Hantgan & Hermans, 1979). The authors concluded from these studies that fibril size was determined primarily by the kinetics of polymer formation and not by the equilibria of the assembly steps. The relationships reported here between the kinetics of fibrin polymer formation and increases in the turbidity of fibrin gels are in agreement with these reports. We also observed that HA and CS accelerted the formation of fibrin and that these changes correlated with an increase in fibrin gel turbidity which also suggest an increase in the diameter of fibers formed.

The effects of HA and CS on fibrin formation may be significant in hemostasis under normal and pathophysiological conditions. For example, patients with rheumatoid arthritis (Laurent & Hallgren, 1985), scleroderma (Laurent et al., 1985a), sarcoidosis (Hallgren et al., 1985), and a variety of

[2] R. D. LeBoeuf, R. R. Gregg, P. H. Weigel, and G. M. Fuller, unpublished results.

[3] R. D. LeBoeuf, R. R. Gregg, G. M. Fuller, and P. H. Weigel, unpublished results.

liver diseases (Frebourg et al., 1986; Laurent et al., 1985b) have elevated HA levels in plasma, and HA–fibrin(ogen) interactions could affect normal fibrin(ogen) metabolism and its participation in hemostasis. Second, the activation of the coagulation cascade and the subsequent hemostatic events are normal consequences of vascular endothelial tissue damage and results in the deposition of fibrin at the site of injury on or near basal lamina GAGs. Fibrin(ogen) interactions with the various components of the basal lamina may play an important role in the rate at which fibrin is formed and may determine, in part, the structure of fibrin networks at the site of tissue injury. The results presented in this study also suggest some important new considerations concerning fibrin formation, namely, that fibrin is the initial matrix that is remodeled during the early stages of tissue repair and that the direct interaction between fibrin and carbohydrate polymers at the site of tissue injury not only may influence fibrin formation but also may be important in directing, regulating, or stimulating cells involved in the reparative process.

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